



# Survey Manual

United States Department of Agriculture  
Animal and Plant Health Inspection Service  
Plant Protection and Quarantine

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## **2006 *Phytophthora ramorum* National Nursery Survey Protocol**

### **Nursery Survey Objectives**

The objective of the 2006 *Phytophthora ramorum* National Nursery Survey is to determine the distribution of *P. ramorum* in the nursery system the United States. This objective will be accomplished by surveying nurseries at risk of harboring or distributing *P. ramorum* infected plants.

### **Biology of *Phytophthora ramorum***

The biology of *P. ramorum* is described in Appendix A. Please read the appendix before starting surveys.

### **Definitions**

HAP: Host and associated host plants listed on the official APHIS List of Hosts and Plants Associated with *Phytophthora ramorum*.

Nursery: Any location where nursery stock is grown, propagated, stored, or sold, or any location from which nursery stock is distributed.

Nursery Dealer: Nurseries that are resellers – wholesale or retail – of nursery plants

Nursery Grower: Nurseries that grow nursery stock

### **Selecting Nurseries to Survey**

States shall

- Inspect all trace back nurseries that were identified in the previous year but were not found positive for *P. ramorum*.
- Inspect a representative sample of nurseries that received plants from known infected suppliers (i.e., trace forward nurseries) and were not found positive for *P. ramorum*.
  - Inspection of all nurseries that received potentially infected stock in the previous year is highly recommended.
  - States may select a sample of nurseries with a history of exposure to *P. ramorum* in order to make better use of available resources, including time, money, and staff. A representative sample should include a subset of nurseries by:
    - Type (dealer or grower)
    - Type of HAPs (particularly those in the six genera believed to be most susceptible to *P. ramorum* infection in nursery settings: *Camellia*, *Rhododendron*, *Viburnum*, *Pieris*, *Kalmia*, and *Syringa* – see next section.)

- Size (number of HAPs in inventory)
- Location (region with favorable conditions for *P. ramorum*; should taking into account microclimates that can exist in nurseries)

If funding permits, states should inspect 20 additional nurseries based on the criteria described below.

- Prioritize the selection of these nurseries based on the following criteria (not in priority order)
  - Never been inspected
  - Not inspected during the previous year
  - Large inventory of HAP (particularly those in the six genera believed to be most susceptible to *P. ramorum* infection in nursery settings.: *Camellia*, *Rhododendron*, *Viburnum*, *Pieris*, *Kalmia*, and *Syringa* – see nest section.)
  - Receives host plants from areas known to be at an elevated risk for *P. ramorum* such as California, Oregon, Washington, British Columbia, and Europe
  - Location (regions with favorable conditions for *P. ramorum*; take into account microclimates that can exist in nurseries)
- Balance your selection between
  - Nursery growers
  - Nursery dealers

Positive national survey, trace forward and trace back sites from the previous two years will be surveyed as part of the follow-up to the confirmed nursery protocol and are not part of the National Survey.

Currently, California, Oregon, and Washington must inspect all interstate shipping nurseries. These inspections met the minimum requirements of the national survey and may be used to satisfy the conditions of this national survey plan; these states may inspect additional qualifying nurseries not required to be surveyed by APHIS.

## Selecting Plants to Inspect

Inspect plants on the official APHIS List of Hosts and Plants Associated with *Phytophthora ramorum* (see Appendix B). The complete and up-to-date host list is available at the APHIS *P. ramorum* Web site. You should consult it before beginning your inspections.

Several years of experience with *P. ramorum* in nurseries now indicates that six plant genera appear to have a high susceptibility to infection by *P. ramorum* in U.S. nursery settings. Inspect HAP and other plants in these genera. For other genera, inspect HAP plants only. The high risk genera are:

- *Camellia*

- *Rhododendron* (excluding azaleas)
  - Camellias and *Rhododendron* are the most commonly reported nursery hosts.
  - Small leaf azalea plants common in nursery trade have never been reported to become infected with *P. ramorum*.
- *Viburnum*
- *Pieris*
- *Kalmia*
- *Syringa* (lilac)

## Timing Nursery Surveys

Nurseries should be surveyed at a time when the best expression of symptoms due to *P. ramorum* is expected.

- Research suggests that the most favorable climate for the expression of symptoms includes ambient temperatures between 3° C and 28° C (optimum 20° C) and free moisture present on host tissue for at least 12 hours over 10 or more days. In many areas this will be in the spring and fall.
- Greenhouses, hot houses and nursery beds under shade cloth or overhead irrigation should be considered micro-climates where optimum conditions are governed more by nursery and market practices than external conditions. Take these micro-climates into consideration when selecting sites and times to survey.
- Disease expression typically begins between 30 and 90 days after bud-break. In many locations, the survey should begin after the spring flush is underway, when some of the leaves have fully expanded. Plants can express symptoms throughout the growing season, though isolation of the pathogen from diseased tissue may be more difficult during hot and dry periods.

## Preparing to Survey Nurseries

Inspectors should receive training in identifying symptoms associated with *P. ramorum* on host plants. At a minimum they should review photographs of the wide range of symptoms possible before starting the survey. Photographs of typical and atypical symptoms are available in Appendix D.

**Remember that symptoms of *P. ramorum* are often not “typical” and over reliance on identification by these symptoms could result in infected plants remaining undetected – the greatest chance of detecting *P. ramorum* infections is through the collection of ANY unhealthy looking plant tissue for laboratory analysis.**

Additional images of *P. ramorum* symptoms are available at the APHIS *P. ramorum*, USDA *Phytophthora ramorum* Educate to Detect (PRED) Program, and California Oak Mortality

Task Force (COMTF) web sites.

- APHIS: <http://www.aphis.usda.gov/ppq/ispm/pramorum/>
- PRED: <http://www.ncpmc.org/alerts/suddenoakdeath/pred.cfm>
- COMTF: <http://www.suddenoakdeath.org>

The University of California at Davis has produced a Nursery Guide for Diseases of *Phytophthora ramorum* on Ornamentals: Diagnosis and Management that is worth downloading, printing out, reviewing, and taking to the field. It is available at:

- UC Davis: <http://anrcatalog.ucdavis.edu/pdf/8156.pdf>

If available, obtain and review an inventory and a location map of host plants in the nursery to help determine where the plants to be inspected are located.

Prior to beginning the inspections, conduct a visual assessment of the nursery as a whole. During this survey identify any low lying areas, standing water, the nursery layout, the general condition of the plants and nursery environment. Use this information to help guide your inspection

### **Determining the Number of Plants to Inspect**

Visually inspect a minimum of number of host plants in each nursery at random based on Table 4. At the discretion of the inspector, more plants may be visually inspected and sampled if conditions suggest this is needed.

**Host plants in the six highly susceptible HAP genera listed previously should be inspected, by genus, at the rates listed in the table. All other host plants are inspected, as a whole, at the rate specified in the table.**

For example, if a nursery has 3,000 camellias, 6000 rhododendron and 8000 plants in host genera other than the six highly susceptible genera minimally inspect 1,055 camellias, 1,087 rhododendrons and 1,087 randomly selected other HAP plants.

<p>Table 4. Use this table determining the number of hosts and associated plants for visual inspection within a nursery. Host plants in the six highly susceptible HAP genera listed previously should be inspected, by genera, at the rates listed in the table. All other host plants are inspected, as a whole, at the rate specified in the table. The numbers below are the minimum number of host and associated host plants that must be inspected in a nursery to ensure detection at a 95% confidence level when the disease is present in 0.5% of the plants, when 75% of infected plants express symptoms.</p>
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Host and Associated plants per nursery	95% Confidence Limit of detecting 0.5% disease
n<500	All plants
501<n<1,000	842
1001<n<5,000	1055
5,001<n<10,000	1087
n>10,001	1115

## Identifying Other Areas of the Nursery to Inspect

Cull Piles: Locate and inspect cull piles of plant materials that have been discarded or taken off sale. Sample if symptomatic plant tissues are observed. Inspect culls pile after you have completed inspections of the rest of the nursery.

## Inspecting the Nursery and Collecting Samples

There are two basic principals should govern the inspection and sampling processes.

1. ***P. ramorum* cannot be diagnosed by a visual inspection of symptoms alone, only laboratory testing can provide a definitive diagnosis.**
2. **If there is any doubt as to whether the symptoms observed are caused by *P. ramorum*, collect a sample.**

Plants chosen to be inspected should be carefully scrutinized. Foliar symptoms of *P. ramorum* infection are highly variable (see Appendix D) and can range from pinpoint discolorations on the leaf surface to large “V” shaped lesions along the leaf mid-vein. In some hosts (Camellia & Rhododendron) low rates of infection can cause premature leaf drop, yielding infected plants that appear to be asymptomatic. As a result, leaves found in the pot or on the ground below the plant should also be checked for possible symptoms and collected for laboratory analysis.

Collect samples of **any and all** plant tissue that appears unhealthy. If there is a large amount of unhealthy tissue, collect as many samples as needed to fully represent the symptoms seen on a genus/species/variety/block basis. This does not mean sampling every single symptomatic plant, but sampling enough of them in any given block so that you are sure to give the lab the material it needs to make a correct diagnosis. Do not be intimidated by a lack of certainty as to what *P. ramorum* symptoms might look like. Remember, other common *Phytophthoras*, other pathogens and environmental stressors can cause similar symptoms that cannot be identified based on visual inspection.

Do not collect samples from healthy, asymptomatic plants. If no unhealthy plants are observed, note how many healthy HAP plants were inspected.

Each sample should consist of a minimum of five leaves; for vaccinum and other small leaf

hosts collect the terminal last 3 inches of branch tips. Consult the testing laboratory for detailed guidance on sample collection and documentation.

Complete a National Nursery Survey Data Collection Form for each location (Appendix F).

Surveyors may wish to draw a map of the nursery and indicate areas inspected and sampled. This can be very useful if resampling is necessary.

Follow these minimum decontamination procedures between nurseries and between hosts within a nursery.

- Decontaminate all equipment you use to take samples between blocks of nursery stock and before leaving a nursery. Use a spray bottle containing a dilute (10%) bleach solution or a quaternary ammonium solution-over all tools between nursery blocks.
- Brush loose dirt from boots and shoes and then spray boots or shoes with disinfection solution in spray bottle between nursery blocks.

## Collecting Sample Tissue by Symptom Type

### Leaf Spots

- Collect symptomatic leaves.
  - Symptomatic fallen leaves *within the pot* of a symptomatic plant can be included in the sample provided they are not exhibiting extensive decay.
  - For plants with very small leaves or needles, samples can be submitted as twig sections with the leaves attached. In these cases try to ensure that the sample has a total of approx. a 3"x3" leaf surface area.

### Twig Dieback-

- Cut the twigs below the cankered regions (well into healthy tissue).
- Sterilize pruning equipment between samples using a dilute (10%) bleach solution or a quaternary ammonium solution

### Cankers on Boles and Branches of Trees

- Follow procedures in your state for surveying and sampling trees.
- In some states nursery inspectors may sample trees while in other states forestry or other officials may be asked to sample trees.

## Handling and Preparing Samples

- Samples should be bagged in a moisture-retaining container, such as a polyethylene bag to prevent drying. **Do not** add extra moisture to the sample to keep it fresh. The extra moisture will actually speed deterioration of the sample.
- Keep the samples cool (around 3° - 6° C) – place them in foam cooler.



- Mail or deliver the sample as soon as possible to preserve freshness (if mailing use overnight mail).
- Remove gloves and place sample bag in a second protective bag.
- After you have double bagged the sample, complete a lab sample form and attach it to the bag (you may also place the lab sample form inside the second bag this reduces the risk that the form could be detected during shipping):
  - Use the sample submission form required by the receiving lab.
  - For samples going to an APHIS lab, use the PPQ Form 391.
- Always write out the identifying label remarks on the outside of the bag with a permanent marker,
  - Attach labels on the outside of bags since labels inside the bag may deteriorate due to moisture and become illegible.
  - Include on all labels with a permanent marker: time, date, collector's identification number, location of sample site, sample number
- Keep the sample cool and out of the sun (have a foam cooler with cold packs available). **Do not** allow them to dry out or over heat.

## Notifying the Lab

Contact receiving lab and let them know the samples are being sent.

## Reporting Results

After diagnosis, the following information should be reported to NAPIS. A more detailed description of the data elements to assist your data management staff is contained in the *P. ramorum* National Nursery Survey Data Worksheet (See Appendix G) and at <http://ceris.purdue.edu/caps/dentry.html>.

Survey Elements to be Recorded in the NAPIS Database			
Observation Number	Observation Date	Data Source	State-County
EPA Site Code (Crop/Host)	Crop (Host) Life Stage ( <b>optional</b> )	Crop Situation (Nursery Type and Survey History)	Latitude/Longitude (or zip code)
EPA Pest Code	Pest Status	Survey Method	Quantification
Descriptor Units	Total Units Check	Diagnostic Lab	Confirmation Method
Lab Process Date	Survey Method	Sample Number	Zip Code (or Lat/Long)
Notes			

Observation Number: It is important that state cooperators follow the following record format to maintain record integrity within the national program. A twelve character code defined as follows:

	Program	State	Site no.	Host Sequence no.	Sample Sequence no.
<b>Description</b>	Survey Code	WA	Nursery Visited: 001 to zzz	01 to zz	01 to zz
<b>Example</b>	SOD	WA	135	35	01

See a complete definition of Observation Number on the NAPIS worksheet.

The management and flow of new survey information collected should

<b><u>Begin with these staff:</u></b>	<b><u>and end after:</u></b>
Surveyors	Standard survey record completed
Survey Coordinators	Survey records collected & reviewed for quality assurance
Data Entry personnel	Data of survey records are entered to NAPIS input buffer
DB Manager	Data records reviewed for quality assurance & accepted

The survey records must be entered into NAPIS according to the CAPS guidelines for pest survey and data submission on a **bi-weekly basis** at each stage of the state survey and laboratory analysis of the results. **New confirmed finds must be reported with 48 hours.**

As data is submitted, a national report will be generated and posted for information on the website.

### **Points of Contact for the 2006 *P. ramorum* National Nursery Survey**

This survey is being coordinated nationally by:

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